

RPP:135D US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Molly F. Kulesz-Martin Art Unit: 1642
Serial No: 08/644,289 Confirmation No: 4031
Filed: May 10, 1996
Examiner: M. Davis
For: p53as PROTEIN AND
ANTIBODY THEREFOR

DECLARATION UNDER 37 C.F.R. 1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Molly F. Kulesz-Martin declares:

1. That she is an inventor in the above-identified patent application.
2. That she has a Ph.D. from SUNY Buffalo - Roswell Park Cancer Institute with concentrations in Cell Biology and Immunology.
3. That she has authored or co-authored over thirty peer reviewed and published papers in the area of p53, DNA binding, gene expression and/or carcinogenesis.
4. That she is the sole inventor of U.S. Patent 5,688,918 granted November 18, 1997 entitled "p53as Protein and Antibody Therefor".
5. That she is intimately familiar with the functions of both p53as referred to in the above-identified patent application and p53-M8.

6. That in view of work done under her direction and supervision, and based upon knowledge known to those skilled in the art, differences between p53as and p53-M8 can be stated as follows:

- a) DNA binding is the most important property of p53 protein. She has demonstrated that p53as is an active DNA binding protein (*EMBO*, 13: 4823-4830, 1994). p53-M8 was found to be inactive in DNA binding by Rotter's group (*Nucleic Acids Res.* 19: 5191-5198, 1991).
- b) Transformation suppression is the major function of wild type p53. In a co-transformation study, p53-M8 showed oncogenic activity to enhance malignant transformation of rat primary embryonic fibroblast (*Oncogene*, 3: 313-321, 1988). She has demonstrated that p53as is a tumor suppressor based on its ability to repress colony formation of Saos-2 cells (*PNAS*, 94: 8982-8987, 1997).
- c) Tetramer formation of p53 is required for its function. By gel filtration analysis of oligomerization of *in vitro* translated p53 proteins, p53-M8 protein has been shown to form monomers or dimers but not tetramers (*EMBO*, 11: 3513-3520, 1992). Using a similar approach, she found that p53as forms tetramers as does regularly spliced p53 does (*EMBO*, 13: 4823-4830, 1994).
- d) Localization of p53 in the nucleus is critical for its function as a transcription factor. By immunofluorescence staining, p53-M8 was localized in both nucleus and cytoplasm whereas regularly spliced wild type p53 was localized in the nucleus (*Mol. Cell. Biol.*, 10: 6565-6577, 1990). She has found that p53as was exclusively localized in the nucleus by indirect immunofluorescent staining with

our anti-p53as antibody (Mol. Cell. Biol., 14: 1698-1708, 1994) and, by observing cells expressing GFP (green fluorescent protein) fused to the N-terminus of wild type p53as.

e) That she has found that the p53as has the sequence:

P53AS	1	MTAMEESQSD	ISLELPLSQE	TFSGLWKLLP	PEDILPSPHC	MDDLILLPQDV	50
P53AS	51	KEFFEGPSEA	LRVSGAPAAQ	DPVTETPGPV	APAPATPWPL	SSFVPSQKTY	100
P53AS	101	QGNYGFHLGF	LQSGTAKSVM	CTYSPLNKL	FCQLAKTCPV	QLWVSATPPA	150
P53AS	151	GSRVRAMAY	KKSQHMTEVV	RRCPHHERCS	DGDGLAPPQH	LIRVEGNLYP	200
P53AS	201	EYLEDRTFR	HSVVPYEPP	EAGSEYTTIH	YKYMENSSCM	GGMNRRLPIL	250
P53AS	251	ITLEDSSGN	LLGRDSFEVR	VCACPGRRR	TEENFRKKE	VLCPELPPGS	300
P53AS	301	AKRALPTCT	ASPPQKKKPL	DGEYFTLKIR	GRKRFEMFRE	LNEALELKDA	350
P53AS	351	HATEESGDSR	AHSSLQPRAF	QALKEESPN	381		
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f) That p53-M8 has a sequence similar to the above except that p53-M8 has a Phe at position 132 (nucleotides 396-399, Arai, et al., Molecular and Cellular Biology, Sept. 1986, p 32-36) and p53as has a Cys at position 142 and the sequences are thus different.

g) That the above facts demonstrate that p53as and p53-M8 have different sequences and function.

- b) That she further declares that all statements made herein of her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statement were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: 1/2/03

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